SOIL SCIENCE

VOLUME 106 October 1968 NUMBER 4

VEGETABLE TANNINS, THEIR DECOMPOSITION AND EFFECTS ON DECOMPOSITION OF SOME ORGANIC COMPOUNDS

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Received for publication November 27, 1967

Tannins have particular significance in both soils and plants because of their ability to combine with proteins to produce resistant complexes and to inhibit certain potential phytopathogens. They participate in formation of soil humus and development of soil profiles (7, 11, 17, 18, 19, 22, 36). High content of tannins in plant materials is associated with resistance to microbial attack (32), and the durability of certain long lived trees and their wood has been ascribed to their high tannin content (24, 29, 36, 37, 38). The tannins are large molecular weight polyphenols that differ in structure and biological significance. They are the fourth most abundant plant constituents following cellulose, hemicelluloses, and lignins and, together with lignin, are the most abundant and widely distributed phenolic polymers of higher plants (34, 36).

Tannins vary in their susceptibility to microbial attack and are transformed slowly by certain fungi, principal among which are species of the genera *Penicillium* and *Aspergillus* (12, 13, 16, 21, 28, 33). Species of *Fusarium, Cylindrocarpon, Gliocladium,* and *Endothia* have been implicated also in the decomposition of various tannins (3, 14, 20). There is little information about decomposition of tannins by basidiomycetes (10, 17). However, these fungi are suspect because of their ability to break down lignins which contain polyphenolic residues similar to those found in tannins (8, 9). Results of Basaraba (1) suggest that some bacteria attack chestnut and wattle tannins.

Hydrolyzable tannins are believed to be more susceptible to microbial attack than condensed tannins (26). A purified preparation of wattle tannin, a representative of the latter, was decomposed very slowly by a mixed soil population in culture solution; from 3 to 17 per cent of the tannin carbon was released as CO_2 aerobically in 21 days, depending on the pH and tannin concentration (4). Under the same conditions two-thirds or more of the carbon of proteins, hemicelluloses, and polysaccharides was oxidized to CO_2. Somewhat more rapid decomposition of tannins of chestnut wood, wattle, and gall nuts was reported by Martin (25).

Protein-tannin complexes are much more resistant to microbial decomposition than unaltered proteins (3, 4, 5, 6, 11, 17, 27) and the complexes were found to be more resistant at pH 4.0 than at neutrality (3, 4, 6). Seldom have the microorganisms involved in the decomposition been determined. Handley (17) implicated wood-rotting basidiomycetes in decomposition of complexes of gelatin and tannins of leaf extracts.

Inhibition of decomposition of some high molecular weight non-proteins was ascribed to inactivation of exocellular microbial enzymes (5), adsorption of tannins by the compounds (6), and to protection of the compounds by coatings of tannin-protein complexes (17).

Previous reports by the junior author and associates included results of studies of the decomposition of some crude tannins, purified wattle tanin, diverse tannin-protein complexes, and mixtures of tannins, polysaccharides, and hemicelluloses in culture solutions (3, 4, 6). This report deals with the decomposition of similar materials in a forest soil.

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MATERIALS AND METHODS

The following tannins were used: tannic acid (52.2% C), gallotannin (53.7% C), chestnut tannin (56.7% C), catechin (61.9% C), wattle tannin (55.4% C), and canaigre tannin (51.4% C). The first three are hydrolyzable tannins and the others condensed tannins.[2] The chestnut, wattle, and canaigre tannins were purified by the method of Roux as modified by Benoit *et al.* (4).

Determinations were made of the decomposition of the tannins and other organic compounds in A-horizon material of a hardwood forest soil of pH 4.2. The soil was a loamy sand that contained 4 per cent organic matter. Fifty gram portions of the soil were added to 300 ml. Florence flasks, and 0.05 g. NH_4Cl was added. In the experiments on decomposition of tannins and tannin complexes, 0.5 g. portions of the finely powdered dry substances were mixed uniformly with the soil. In the experiments on the influence of tannins on decomposition of non-proteins, 0.5 g. portions of these compounds (chitin, saponin, starch, pectin) were used and the tannins were added separately in amounts to provide concentrations of 0.1, 0.4, and 1.0 per cent. The results are reported on the basis of 100 g. portions of soil and percentages of the substances incorporated with the soil. The moisture-holding capacity of the soil was brought to 60 per cent and the flasks of soil were incubated at 28°C for 60 days during which the amounts of CO_2 produced were determined (35). The values for CO_2 produced from control soils were subtracted from those of the amended soils.

The tannin-protein complexes were prepared as follows: 5% (w/v) solutions of each of 5 tannins (tannic acid, gallotannin, catechin, chestnut tannin, wattle tannin) and four proteins (albumin, Na-caseinate, gelatin, gliadin) were prepared in 0.5% NaCl and held at room temperature for several hours. The tannin solutions were adjusted to pH 3.0. Equal volumes of solutions of tannin and protein were slowly combined and then mixed in a rotary shaker for 1 hr. The flocculent gummy precipitates were filtered off, washed, and dried at 100°C, and the horny resinous products were ground to fine powders. In this manner, 20 different complexes were prepared. The protein contents of the tannin-protein complexes were calculated from their nitrogen contents. The carbon contents of the organic compounds and the CO_2-carbon produced during their decomposition were determined. Percentage decomposition of the compounds refers to the percentage of the carbon content recovered as CO_2. Inhibition of protein decomposition was calculated from the amounts of CO_2 produced in soil from each of the organic compounds used separately (e.g. tannic acid, albumin) and from the synthesized complexes. Inhibition of decomposition of other organic substances was calculated as follows, using the gallotannin-chitin item as the example. An appropriate amount of gallotannin was added to one portion of soil (A), and of chitin to another (B). The same amounts of gallotannin and chitin were mixed separately with a third portion of soil (C). The CO_2 produced by the three portions of soil was determined. Inhibition of decomposition refers to the decrease in amount of CO_2-carbon released from soil C from the sum of the amounts from soils A and B.

RESULTS

Decomposition of tannins

Four of the six tannins underwent appreciable decomposition in the forest soil in 60 days (fig. 1). This included the three hydrolyzable tannins, tannic acid (44%), gallotannin (40%), and chestnut tannin (26%), and one of the condensed tannins, catechin (27%). Very little CO_2 was released from wattle and canaigre tannins. With all tannins decomposition was most rapid during the first 12 days.

Decomposition of tannin-protein complexes

All of the complexes of the tannins with the proteins albumin, casein, gelatin, and gliadin contained more protein than tannin. The tannin-protein ratios varied from 0.49:1 to 0.96:1 and 80 per cent of the ratios were between 0.64:1 and 0.89:1 (table 1). All of the complexes decomposed somewhat and there was considerable variation in susceptibility to attack of the complexes of any one tannin. In general, the resistance of the complexes to decomposition and the inhibitive effects of the tannins on decomposition paralleled decomposability of the tannins (fig. 1); the most readily decomposed complexes were those containing tannic acid, gallotannin, and catechin. The complexes of high molecular weight tannins, wattle and chestnut tannins, were the most resistant to microbial decomposition. The three simpler tannins caused inhibition of protein decomposition greater than 24 per cent in only two cases whereas the inhibitive effect of the high molecular weight tannins was less than 24 per cent in only one of 8 cases.

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[2] Tannic acid, gallotannin, and catechin were highest purity compounds obtained from Fischer Scientific Co., Baker Chemical Co., and Mann Research Laboratories respectively. Dried extracts of crude chestnut tannin, wattle tannin, and canaigre tannin were generously provided by T. C. Cordon of the Eastern Utilization Research and Development Division, Agr. Res. Service, USDA.

As with the tannins, the tannin-protein complexes decomposed in soil most rapidly during the first few days of incubation. The rate of decomposition toward the end of the incubation period of 60 days was very slow which may indicate that the residue was of a resistant nature.

Influence of tannins on decomposition of some non-proteins

When several non-proteins were mixed with the forest soil and incubated for 60 days the following amounts of the substrate carbon were released as CO_2: chitin, 50%; saponin, 42%; starch, 37%; pectin, 38%. As shown by table 2, tannins were generally effective in reducing the decomposability of non-protein substrates even though the tannins and the other compounds were added to the soil separately and not as preformed complexes, as was the case with the tannins and proteins. Gallotannin, chestnut tannin, and catechin had the most consistent and greatest inhibitive effects on the decomposition of chitin, saponin, and starch. Except for their effects on pectin and the effect of wattle tannin on saponin, the average inhibitions by the tannins were nearly alike. Decomposition of pectin was affected least, with only catechin and high concentrations of gallotannin causing significant inhibition of decomposition. With few exceptions, inhibition of decomposition increased with increasing concentrations of tannin, but the differences were not great for ten-fold increases in tannin. In some instances, 0.1% tannin was as effective as 1.0% tannin as an inhibitor of decomposition.

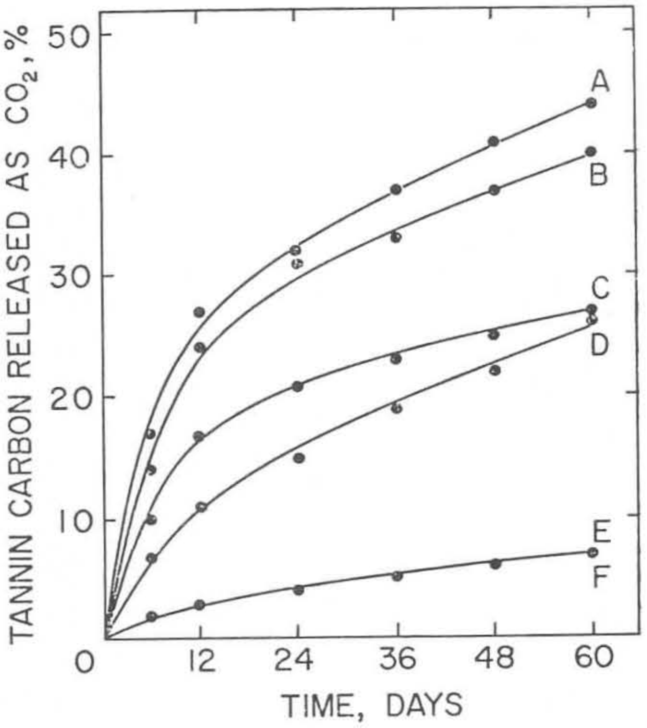

FIG. 1. Decomposition of tannins in a forest soil. A—tannic acid, B—gallotannin, C—catechin, D—chestnut tannin, E—wattle tannin, F—canaigre tannin.

DISCUSSION

Previous reports of this series were concerned with decomposition of tannins and their complexes in culture solutions whereas this communication deals with their decomposition in soil. In some respects these results differ from what might have been expected from the results with the culture solutions. The differences are ascribed to various effects of the soil on the organic compounds, such as decreasing their mobility and distribution, affecting their concentration in the soil water and reactivity by adsorption and chemical reaction with soil minerals, and direct influences of the heterogeneous soil system on the microorganisms. There is evidence that soil minerals affect tannins; it was observed by Scheffer *et al.* (30, 31) that iron oxides and silicic acids catalyzed oxidative polymerization

TABLE 1

Decomposition of tannin-protein complexes

Complex*	Tannin-Protein Ratio	C Content of Substrate	CO$_2$-C Produced			Inhibition of Decomposition
			Complex		Complex Components used Separately	
		mg	*mg*	%	*mg*	%
Ta-albumin	0.69:1	497	204	41	232	12
Ta-casein	0.89:1	464	221	48	235	6
Ta-gelatin	0.67:1	471	305	65	308	1
Ta-gliadin	0.89:1	473	220	47	275	20
Ga-albumin	0.64:1	411	264	64	266	1
Ga-casein	0.82:1	453	210	46	273	23
Ga-gelatin	0.96:1	435	264	61	314	16
Ga-gliadin	0.75:1	456	196	43	310	37
Ch-albumin	0.79:1	470	90	19	175	49
Ch-casein	0.89:1	459	152	33	200	24
Ch-gelatin	0.67:1	446	93	21	268	65
Ch-gliadin	0.67:1	483	80	17	264	70
Ca-albumin	0.72:1	462	214	46	222	4
Ca-casein	0.49:1	491	234	48	243	4
Ca-gelatin	0.82:1	451	168	37	289	42
Ca-gliadin	0.92:1	494	208	42	273	24
Wa-albumin	0.89:1	551	99	18	183	46
Wa-casein	0.64:1	496	116	23	219	47
Wa-gelatin	0.49:1	492	230	47	282	18
Wa-gliadin	0.52:1	526	150	29	267	44

* Ta—tannic acid, ga—gallotannin, ch—chestnut tannin, ca—catechin, wa—wattle tannin.

TABLE 2

Inhibition of decomposition of non-proteins in association with tannins

Tannin		Substrate + Tannin C Evolved as CO$_2$-C				Decrease in CO$_2$-C Evolved over Constituents used Separately			
		Chitin	Saponin	Starch	Pectin	Chitin	Saponin	Starch	Pectin
	%	%	%	%	%	%	%	%	%
Gallotannin	0.1	43	37	24	39	14	12	37	1
	0.4	37	28	25	33	24	36	38	21
	1.0	30	25	20	29	33	39	49	25
Chestnut	0.1	37	32	24	37	24	22	35	3
	0.4	28	25	19	31	29	25	36	1
	1.0	23	19	19	30	39	41	37	4
Catechin	0.1	35	25	25	28	25	38	30	23
	0.4	29	23	23	26	29	34	29	23
	1.0	26	22	21	21	28	34	31	34
Wattle	0.1	34	35	27	36	28	13	25	3
	0.4	24	27	22	28	38	14	23	1
	1.0	16	18	15	19	38	15	22	5

of polyphenols, and Kyuma and Kawaguchi (23) noted that allophane in clays adsorbed large amounts of chestnut tannin and apparently promoted its oxidative polymerization.

As noted by others, hydrolyzable tannins, including the chemically complex chestnut tannin, decomposed more rapidly than condensed tannins, and the more complex the compounds of each group the more resistant they were. In general, also, resistance of the tannin-protein complexes to microbial decomposition increased with increase in chemical complexity of the tannin component. This is consistent with the observations that complexes of gallotannin and enzyme proteins decomposed rapidly (27) whereas complexes of gelatin and condensed tannins were considerably more resistant, particularly at acid reactions (2, 4).

An acid forest soil (pH 4.2) was used in all of the experiments. Since it has been found that the tannin-protein complexes are more resistant at acid reactions they might have been less resistant in more nearly neutral field soils.

Under the experimental conditions used, the effects of the tannins on decomposition of relatively large molecular weight non-proteins were not consistent with their degree of complexing of proteins and the stability of the tannin-protein complexes. All four tannins significantly reduced decomposition of chitin and starch and, with the exception of wattle tannin, also that of saponin. Whereas in other experiments chestnut and wattle tannins were most effective in inhibiting protein decomposition, both tannins were least effective in the inhibition of pectin decomposition, and wattle tannin only slightly inhibited saponin decomposition. It is presumed that the principal tannin effect involves complexing of the microbial enzymes concerned with initial hydrolyses of the compounds. In soil this complexing would have been affected by mobility of the tannins and the other compounds as well as by other factors that influence contact between the tannins and enzymes. The results might have been different if the tannins and the other non-protein compounds had been mixed in solution before they were added to the soil. In this connection it has been shown that, with mixtures of wattle tannin and pectin in solution, the inhibitive effect of the tannin in 21 days was 17 per cent at pH 4.0 and 25 per cent at pH 7.0 (6), whereas it was only 3 per cent in the forest soil in 60 days.

Under conditions in nature the tannins and proteins and other materials with which they combine may react within the plant tissues to form resistant complexes (17, 18). In the present study, tannin-protein complexes were used to simulate such conditions. However, when the tannins and the other compounds were incorporated with the soil separately and not as performed complexes, conditions seem to have been somewhat unfavorable for complex formation.

SUMMARY

Decomposition of tannins, tannin-protein complexes, and some non-proteins in the presence of tannins in a forest soil was estimated from the amounts of CO_2 produced in 60 days. The following was the order of increasing decomposability of the tannins: wattle and canaigre tannins, chestnut tannin and catechin, gallotannin and tannic acid. Resistance of the tannin-protein complexes varied with the tannins and proteins. The average resistance of the gliadin complexes was greater than that of complexes of albumin, casein, and gelatin. The chestnut and wattle tannin-protein complexes were more resistant than those of catechin, tannic acid, and gallotannin. In general, stability of the tannin-protein complexes was a function of the resistance of the tannin portion of the complexes; complexes of high molecular weight tannins were most resistant to microbial decomposition.

When tannins were incorporated with soil to which chitin, saponin, starch, or pectin had been added, decomposition of the latter compounds was appreciably reduced in most cases. The greater the amount of tannin the greater was the inhibitive effect but it was not proportional to the amount of tannin. The tannins inhibited decomposition of starch, chitin, and saponin more than that of pectin. The average inhibitive effect of catechin exceeded that of gallotannin and chestnut tannin. Wattle tannin had the least effect.

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